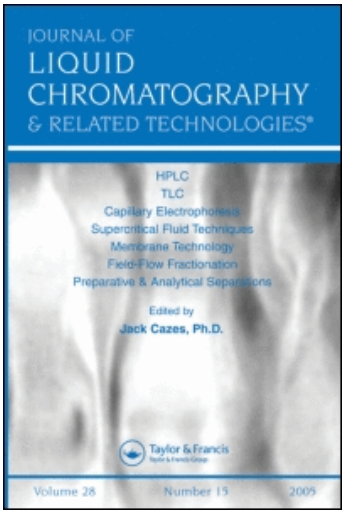

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METHODS FOR ASSAY AND RELATED SUBSTANCES IN NITRENDIPINE RAW MATERIALS

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ABSTRACT

A liquid chromatographic method has been developed for the determination of nitrendipine and related compounds in drug raw material. The method resolves the available related compounds from the drug and each other. The limit of quantitation for the related compounds is about 0.05%.

INTRODUCTION

Nitrendipine, an antihypertensive, has not been described in monographs by either the USP or the BP. Development of a method for drug assay

* Correspondence

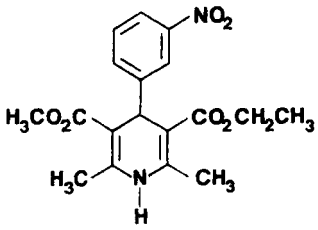
TABLE 1

Nitrendipine and Related Compounds

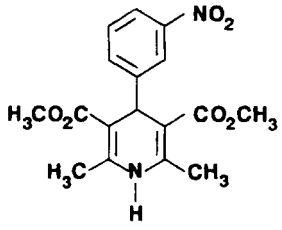
I	3,5-pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-, ethyl methyl ester (nitrendipine)
II	3,5-pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-, dimethyl ester
III	3,5-pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-, diethyl ester
IV	3,5-pyridinedicarboxylic acid, 2,6-dimethyl-4-(3-nitrophenyl)-, ethyl methyl ester

and the determination of related compounds was therefore undertaken. Nitrendipine related compounds available for this work are listed in Table 1 and the structures of the compounds are depicted in Figure 1. II and III are reaction byproducts and IV is a degradation product (1). Nitrendipine is similar in structure to nifedipine, which is 3,5-pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-, dimethyl ester.

An HPLC method appeared during the development of the present method (2). It separates impurities II and III from the drug but there is no mention of impurity IV, the most probable degradation product. IV forms upon exposure to UV, but not visible, light during the time periods typically required for analysis (1).



I Nitrendipine



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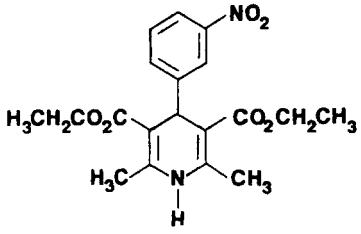
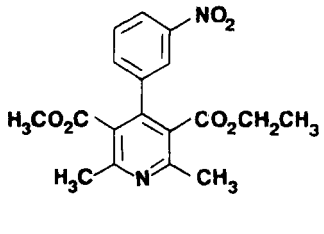


III



IV

Figure 1. Chemical structures of nitrendipine and the available related compounds.

MATERIALS

Chemicals

Nitrendipine and compounds II, III and IV were supplied by Miles Canada; their NMR spectra in deuterated chloroform conform to the respective structures. Acetonitrile and methanol (J.T. Baker Co., Phillipsburg, NJ); ammonium phosphate monobasic (Fisher Scientific, Fairlawn, NJ); tetrahydrofuran, without preservative (Aldrich Chemical, Milwaukee, WI) were HPLC grade. Deionized water was used.

Equipment

The HPLC systems (Varian 5560 or Varian 5060) were fitted with a 10-µL loop (Rheodyne injector #7126), a UV detector (Varian UV-100 or UV-200) set at 235 nm, autosamplers (Varian 8085 or Spectra Physics SP8780 XR) and data stations (Varian Vista 650). Poly(octadecylsilane) columns, 4-µm, 3.9 X 150 mm, (Waters, Novo-Pak C-18, serial Nos T00731 R11 and T81672) were used at ambient temperature with a mobile phase flow rate of 1.5 mL/min. Other equipment was as follow: UV/VIS spectrophotometer - Varian DMS 90 connected to a HP 85 computer with plotter and disk drive.

METHOD

Mobile phase

Tetrahydrofuran (preservative free): acetonitrile: buffer (5:35:60 V/V). The buffer was prepared by adding 0.05 M ammonium hydroxide to 0.05 M ammonium phosphate monobasic to obtain a final pH of 5.0.

Solutions

The following solutions were prepared in methanol and protected from light at all times: resolution solution (0.01 mg/mL each of nitrendipine and IV); related compounds standard solution (0.002 mg/mL nitrendipine); related compounds test solution (1.0 mg/mL nitrendipine, accurately known); assay standard and test solutions (0.1 mg/mL nitrendipine).

System Suitability

A 10 µL aliquot of the resolution solution was injected into the chromatograph. The system was deemed suitable for use if the resolution between nitrendipine and IV was greater than 2.5, the efficiency of the column was not less than 25,000 plates/meter, the tailing factor was less than 1.5 and six aliquots of the resolution standard solution gave a relative standard deviation of not more than 10%, all calculated from the nitrendipine peak using USP procedures (3). For drug assay, suitability was established if five 10-µL aliquots of the assay standard solution gave a coefficient of variation of less than 1%.

Procedure

Related compounds - Aliquots (10 µL) of the related compounds standard and test solutions were injected into the chromatograph and run for thirty minutes. The percentage of each impurity was calculated from $[100\ (A_i/A_s)(C_s/C_u)]$, where A_i is the peak area response due to each individual impurity, A_s is the peak area response due to nitrendipine in the related compounds standard solution and C_s and C_u are the concentrations of nitrendipine in the related compounds standard and test solutions, respectively.

Assay - Aliquots (10 µL) of the assay standard and test solutions were injected into the chromatograph and run for 15 minutes. The percentage of nitrendipine was calculated from

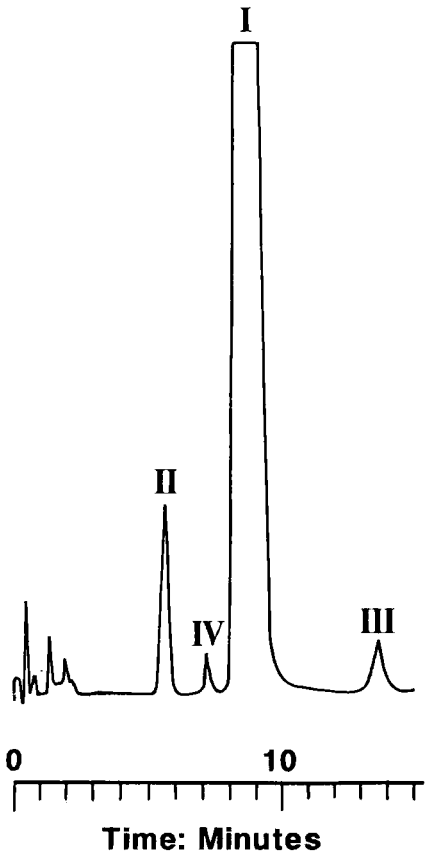


Figure 2. Chromatogram of nitrendipine in the presence of its related compounds. The amounts on column were: nitrendipine (10.21 µg), II (0.024 µg), III (0.020 µg) and IV (0.022 µg).

$[100(A_t/A_r)(C_r/C_t)]$, where A_t and A_r are the areas of the nitrendipine peaks due the assay test and assay standard solutions, respectively, and C_t and C_r are the respective concentrations.

TABLE 2
UV and HPLC Characteristics of Nitrendipine and Related Compounds

Compound	UV absorbance			HPLC	
	Conc[1]	Maxima	Abs[2]	Resp[3]	RRT[4]
I	9.85	204,235,270	1.0	1.0	1.0
II	10.0	205,235,270	0.83	0.99	0.65
III	9.2	205,237,270	0.92	0.99	1.57
IV	9.97	204,215,265	0.52	0.58	0.82

1. Concentration, mg/mL, in methanol.
2. Relative absorptivity at 235 nm.
3. HPLC response relative to nitrendipine for which the slope was 800 area counts /ng.
4. Retention time relative to nitrendipine at about 8.7 min.

RESULTS

Chromatography

A typical chromatogram showing the resolution of nitrendipine from the available related compounds is shown in Figure 2. The UV maxima and relative absorptivity, and the liquid chromatographic responses and relative retention times are given in Table 2. The chromatographic response of all compounds was linear in the range from 6 to 125 ng on column, corresponding to 0.06 to 1.25% relative to nitrendipine in the test solution, with the square of the correlation coefficient greater than 0.997. Coefficients of variation of six injections of the related compounds standard solution (0.002 mg/mL

nitrendipine in methanol) ranged between 2.0 and 7.5%. For the assay standard solution, 0.1 mg/mL, the coefficient of variation was 0.7%.

Ruggedness of the Method

An increase in pH from 5.0 to 5.1 lead to an increase in retention times of about 1 minute. A decrease in acetonitrile in the mobile phase from 35 to 25% resulted in an increase in retention time of 50 min. A decrease of 2% in tetrahydrofuran increased retention times by 2 min. Tetrahydrofuran is essential to obtain resolution of IV from nitrendipine. Changing the flow rate from 1.5 mL/min to 1.0 mL/min increased retention time of all compounds by about 4 min. Peak tailing also increased.

ACKNOWLEDGEMENTS

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